PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference HP1103	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/FI 01/00103	International filing date *(day/month/year)* 6 February 2001	(Earliest) Priority Date *(day/month/year)* 21 March 2000
Applicant HORMOS MEDICAL OY LTD et al		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of the sequence listing:

   [ ] contained in the international application in written form.

   [X] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readable form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

2. [X] Certain claims were found unsearchable (See Box I).

3. [ ] Unity of invention is lacking (See Box II).

4. With regard to the title,

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

   Diagnosis of a person's risk of developing alcoholism.

5. With regard to the abstract,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI01/00103

Box I **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 7
because they relate to subject matter not required to be searched by this Authority, namely:

Claim 7 relate to a method of treatment of the human body by therapy/ Rule 39.1(iv). Nevertheless, a search has been executed for this claim. The search has been based on the alleged effects of the treatment.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 01/00103

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C12Q 1/68, C07K 14/575, A61K 48/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C12Q, G01N, C07K, A61K, C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	Alcoholism: clinical and experimental research, Volume 24, No 5, 2000, T. Okubo et al, "Polymorphism of the neuropeptide Y gene an association study with alcohol withdrawal" column 35	1-9
	--	
A	WO 9932518 A1 (HORMOS MEDICAL OY LTD.), 1 July 1999 (01.07.99), claims 1-20, abstract	1-9
	--	

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 June 2001	28 -06- 2001
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Lars Wallentin/ELY Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 01/00103

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Alcohol Clin Exp Res, Volume 6, June 1999, Hwang BH et al, "Innate differences of neuropeptide Y (NPY) in hypthalamic nuclei and central nucleus of the amygdala between selectively bred rats with high and low alcohol preference", abstract --	1
A	Alcohol Clin Exp Res, Volume 22, No 8, November 1998, Ehlers CL et al, "Neuropeptide Y levels in ethanol-naive alcohol-preferring and nonpreferring rats and in Wistra rats after ethanol exposure", page 1778 - page 1782, abstract -- --------	1

INTERNATIONAL SEARCH REPORT
Information on patent family members

28/05/01

International application No.
PCT/FI 01/00103

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9932518 A1	01/07/99	AU 1673599 A	12/07/99
		CN 1282338 T	31/01/01
		EP 1037922 A	27/09/00
		NO 20003116 A	18/08/00
		US 6046317 A	04/04/00

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

ÖHMAN, Ann-Marie
c/o Hormos Medical Oy Ltd
Tykistökatu 6 A
FIN-20520 Turku
FINLANDE

Date of mailing (day/month/year) 25 January 2002 (25.01.02)	
Applicant's or agent's file reference HP1103	**IMPORTANT NOTIFICATION**
International application No. PCT/FI01/00103	**International filing date** (day/month/year) 06 February 2001 (06.02.01)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
HORMOS MEDICAL OY LTD Tykistökatu 6 A FIN-20520 Turku Finland	FI	FI
	Telephone No. +358 2 333 7697	
	Facsimile No. +358 2 333 7690	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [X] the address [ ] the nationality [ ] the residence

Name and Address	State of Nationality	State of Residence
HORMOS MEDICAL CORPORATION Itäinen Pitkäkatu 4 B FIN-20520 Turku Finland	FI	FI
	Telephone No. +358 2 3337697	
	Facsimile No. +358 2 3337690	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Céline Faust
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994) 004620328

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 09 October 2001 (09.10.01)	
International application No. PCT/FI01/00103	**Applicant's or agent's file reference** HP1103
International filing date (day/month/year) 06 February 2001 (06.02.01)	**Priority date** (day/month/year) 21 March 2000 (21.03.00)
Applicant KAUHANEN, Jussi et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

13 August 2001 (13.08.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Claudio BORTON
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/331 (July 1992)

FI0100103

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

8

Applicant's or agent's file reference HP1103	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/FI01/00103	International filing date *(day/month/year)* 06.02.2001	Priority date *(day/month/year)* 21.03.2000
International Patent Classification (IPC) or national classification and IPC7 C12Q 1/68, C07K 14/575, A61K 48/00		
Applicant HORMOS MEDICAL CORPORATION et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☒ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☒ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
13.08.2001	21.02.2002
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Facsimile No. 08-667 72 88 Telex 17978 PATOREG-S	Authorized officer Lars Wallentin/MP Telephone No. 08-782 25 00

Form PCT/IPEA/409 (cover sheet) (January 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/FI01/00103

I. Basis of the report

1. With regard to the **elements** of the international application:*

☒ the international application as originally filed

☐ the description:
pages _____ , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☐ the claims:
pages _____ , as originally filed
pages _____ , as amended (together with any statement) under article 19
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☐ the drawings:
pages _____ , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☐ the sequence listing part of the description:
pages _____ , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheet/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2 (c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item I and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/FI01/00103

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1).

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

The priority is considered valid, therefore document "Alcoholism: clinical and experimental research, Volume 24, No 5, 2000, T. Okubo et al, "Polymorphism of the neuropeptide Y gene an association study with alcohol withdrawal" is of no relevance.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/FI01/00103

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 1, 4-7

because:

☒ the said international application, or the said claims Nos. 1, 4-7
relate to the following subject matter which does not require an international preliminary examination *(specify)*:

Claims 1 and 4-7 relate to methods of treating the human body by therapy, as well as diagnostic methods. /Rule 67.1 (iv).

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _____
are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for said claims Nos. _____

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	2-3,8-9	YES
	Claims		NO
Inventive step (IS)	Claims	2-3,8-9	YES
	Claims		NO
Industrial applicability (IA)	Claims	2-3,8-9	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The following documents were cited in the International Search Report:

D1: WO 9932518 A1
D2: Alcohol Clin Exp Res, Volume 6, June 1999, Hwang BH et al., "Innate differences of neuropeptide Y (NPY) in hypthalamic nuclei and central nucleus of the amygdala between selectively bred rats with high and low alcohol preference"
D3: Alcohol Clin Exp Res, Volume 22, No 8, November 1998, Ehlers CL et al., "Neuropeptide Y levels in ethanol-native alcohol-preffering and nonpreffering rats and in Wistra rats after ethanol exposure"

Cited document D1 discloses a connection between a leucine 7 to proline polymorphism in the signal peptide part of preproNPY and increased serum cholesterol levels. It is speculated that the polymorphism could impair the synthesis of preproNPY, which subsequently could lead to altered NPY activity (page 16, line 3-18).

Cited document D2 discloses that NPY-knockout mice drink more alcohol than normal mice do and that NPY agonists would enhance NPY function, which could be useful for the treatment of alcoholism.

Cited document D3 shows that NPY may influence the development of alcohol preference.

.../...

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: V.

D2 leads a person skilled in the art to the fact that impaired NPY-function is connected to alcoholism. However, the impairment is not based on polymorphisms. D1 shows one of the possible NPY-altering activities but it does not indicate that the polymorphism may have a connection to alcoholism.

Consequently, it is not regarded as obvious for a person skilled in the art to combine the knowledge of D1 with D2 in order to obtain the invention according to claim 1.

The invention according to claims 1-9 is thus novel and considered to involve an inventive step. It is also considered to be industrially applicable.

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

Application No. Patent No.	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date (valid claim) *(day/month/year)*

Alcoholism: clinical and experimental research, Volume 24, No 5, 2000, T. Okubo et al, "Polymorphism of the neuropeptide Y gene an association study with alcohol withdrawal.

2. Non-written disclosures (Rule 70.9)

Kind of non-written disclosure	Date of non-written disclosure *(day/month/year)*	Date of written disclosure referring to non-written disclosure *(day/month/year)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 01/00103

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C12Q 1/68, C07K 14/575, A61K 48/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C12Q, G01N, C07K, A61K, C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	Alcoholism: clinical and experimental research, Volume 24, No 5, 2000, T. Okubo et al, "Polymorphism of the neuropeptide Y gene an association study with alcohol withdrawal" column 35	1-9
A	WO 9932518 A1 (HORMOS MEDICAL OY LTD.), 1 July 1999 (01.07.99), claims 1-20, abstract	1-9

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 June 2001	28 -06- 2001
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Lars Wallentin/ELY Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 01/00103

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Alcohol Clin Exp Res, Volume 6, June 1999, Hwang BH et al, "Innate differences of neuropeptide Y (NPY) in hypthalamic nuclei and central nucleus of the amygdala between selectively bred rats with high and low alcohol preference", abstract ---	1
A	Alcohol Clin Exp Res, Volume 22, No 8, November 1998, Ehlers CL et al, "Neuropeptide Y levels in ethanol-naive alcohol-preferring and nonpreferring rats and in Wistra rats after ethanol exposure", page 1778 - page 1782, abstract --- --------	1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI01/00103

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 7
because they relate to subject matter not required to be searched by this Authority, namely:

Claim 7 relate to a method of treatment of the human body by therapy/ Rule 39.1(iv). Nevertheless, a search has been executed for this claim. The search has been based on the alleged effects of the treatment.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

28/05/01

International application No.
PCT/FI 01/00103

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9932518 A1	01/07/99	AU 1673599 A	12/07/99
		CN 1282338 T	31/01/01
		EP 1037922 A	27/09/00
		NO 20003116 A	18/08/00
		US 6046317 A	04/04/00

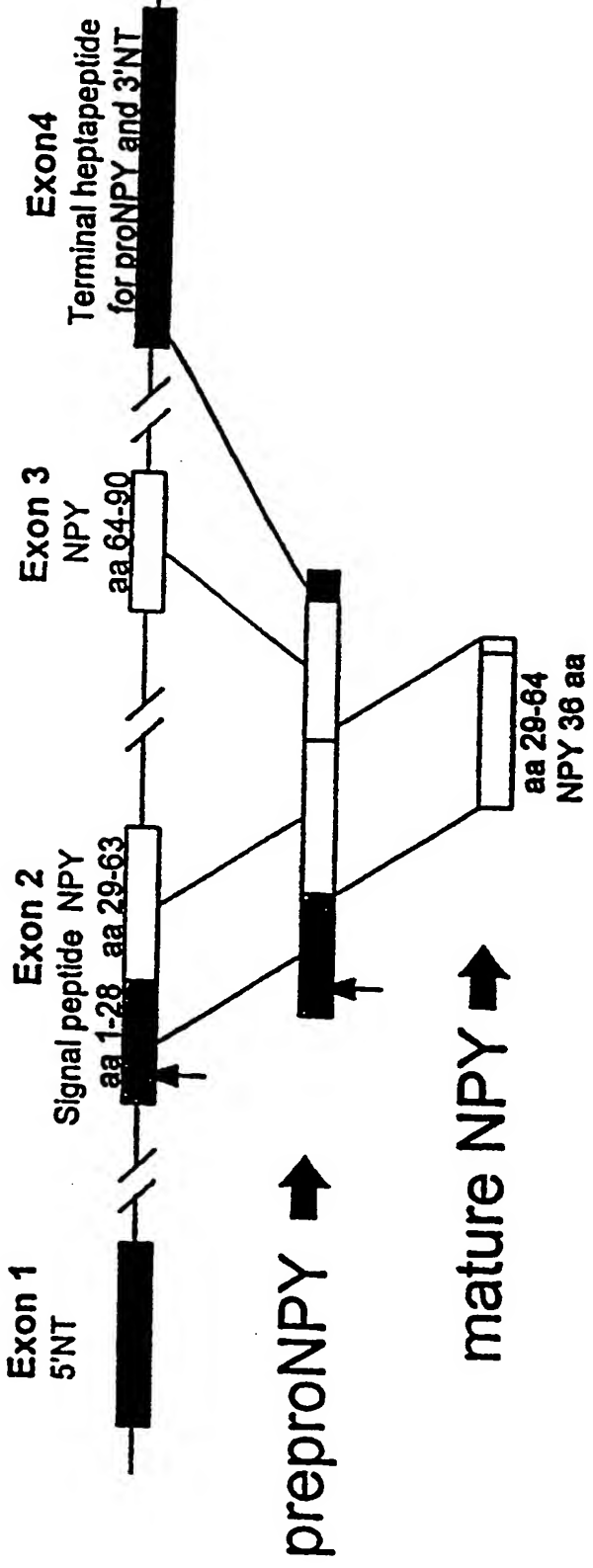

FIG. 1a

HUMAN NEUROPEPTIDE Y (NPY) GENE

EXON 1 (M14295)

```
1   ccgcttcttc aggcagtgcc tggggcggga gggttggggt gtgggtggct ccctaagtcg
61   cactcgtgc ggctgcggtt ccagcccccc ccccccgcca ctcaggggcg ggaagtggcg
121 ggtgggagtc acccaagcgt gactgcccga ggcccctcct gccgcggcga ggaagctcca
181 taaaagccct gtcgcgaccc gctctctgcA CCCCATCCGC TGGCTCTCAC CCCTCGGAGA
241 CGCTCGCCCG ACAGCATAGT ACTTGCCGCC CAGCCACGCC CGCGCGCCAG CCACCGTGAG
301 tgctacgacc cgtctgtcta ggggt
```

EXON 2 (M14296)

```
1   cccgtccgtt gagccttctg tgcctgcagA TGCTAGGTAA CAAGCGACTG GGGCTGTCCG
61  GACTGACCCT CGCCCTGTCC CTGCTCGTGT GCCTGGGTGC GCTGGCCGAG GCGTACCCCT
121 CCAAGCCGGA CAACCCGGGC GAGGACGCAC CAGCGGAGGA CATGGCCAGA TACTACTCAG
181 CGCTGCGACA CTACATCAAC CTCATCACCA GGCAGAGgtg ggtgggaccg cgggaccgat
241 tccggga
```

EXON 3 (M14297)

```
1   acttgcttta aaagactttt ttttttccag ATATGGAAAA CGATCTAGCC CAGAGACACT
61  GATTTCAGAC CTCTTGATGA GAGAAAGCAC AGAAAATGTT CCCAGAACTC Ggtatgacaa
121 ggcttgtgat ggggacattg tt
```

EXON 4 (M14298)

```
1   CCTTACATGC TTTGCTTCTT ATGTTTTACA Ggcttgaaga ccctgcaatg tggtgatggg
61  aaatgagact tgctctctgg ccttttccta ttttcagccc atatttcatc gtgtaaaacg
121 agaatccacc catcctacca atgcatgcag ccactgtgct gaattctgca atgttttcct
181 ttgtcatcat tgtatatatg tgtgtttaaa taaagtatca tgcattcaaa agtgtatcct
241 cctcaatgaa aaatctatta caatagtgag gattattttc gttaaactta ttattaacaa
```

FIG. 1b

HUMAN NEUROPEPTIDE Y (NPY) mRNA

K01911

```
1   accccatccg ctggctctca cccctcggag acgctcgccc gacagcatag tacttgccgc
61  ccagccacgc ccgcgcgcca gccaccatgc taggtaacaa gcgacgggg ctgtccggac
121 tgaccctcgc cctgtccctg ctcgtgtgcc tgggtgcgct ggccgaggcg tacccctcca
181 agccggacaa cccgggcgag gacgcaccag cggaggacat ggccagatac tactcggcgc
241 tgcgacacta catcaacctc atcaccaggc agagatatgg aaaacgatcc agcccagaga
301 cactgatttc agacctcttg atgagagaaa gcacagaaaa tgttcccaga actcggcttg
361  agaccctgc aatgtggtga tgggaaatga gacttgctct ctggcctttt cctattttca
421 gcccatattt catcgtgtaa aacgagaatc cacccatcct accaatgcat gcagccactg
481 tgctgaattc tgcaatgttt tcctttgtca tcattgtata tatgtgtgtt taaataaagt
541 atcatgcatt c
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c

FIG. 1c

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(54) Title: DIAGNOSIS OF A PERSON'S RISK OF DEVELOPING ALCOHOLISM

(57) Abstract: The invention relates to methods for diagnosing a person's susceptibility for having a risk for the development of alcoholism. The invention relates further to methods for treating persons diagnosed for having risk for the development of alcoholism in order to prevent the development of said condition. The invention also concerns methods to investigate or screen pharmaceuticals or genetic aims useful in the treatment of said condition, by using an animal model including a transgenic animal.

Diagnosis of a person's risk of developing alcoholism.

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FIELD OF THE INVENTION

This invention relates to methods for diagnosing a person's susceptibility for having a risk for the development of alcoholism. The invention relates further to methods for preventing or treating persons diagnosed for having risk for the development of alcoholism, in order to prevent the development of said condition. The invention also concerns methods to investigate or screen pharmaceuticals or genetic aims useful in the prevention or treatment of alcoholism, by using an animal model including a transgenic animal.

BACKGROUND OF THE INVENTION

The publications and other materials used herein to illuminate the background of the invention, and in particular, cases to provide additional details respecting the practice, are incorporated by reference.

Neuropeptide Y (NPY) is a hexatriocontapeptide amide that is well characterized as a neuromodulator in the central nervous system [Gray and Morley, 1986; Lundberg et al.,1982]. The best known effects of NPY are stimulation of feeding [Clark et al., 1985; Levine and Morley, 1985; Stanley and Leibowitz 1985] and increased energy storage through lipoprotein lipase activation in white adipose tissue [Billington et al., 1991; Billington et al., 1994]. Recent findings in rodents suggest that NPY may also be a potential regulator of ethanol consumption [Ehlers et al., 1998a; Ehlers et al., 1998b;

Thiele et al., 1998; Cokerill, 1998; Tecott and Heberlein, 1998]. The preference for alcohol seems to be inversely related to NPY levels in brain [Thiele et al., 1998]. NPY-deficient mice show increased consumption of ethanol, whereas transgenic mice that overexpress a NPY gene have a lower preference for ethanol and are more sensitive to its sedative/hypnotic effects [Thiele et al., 1998]. NPY and ethanol have a similar electrophysiological profile [Ehlers et al., 1998b], and both are known to have anxiolytic properties [Thiele et al., 1998; Heilig et al., 1992; Palmiter et al., 1998; Stewart et al., 1993]. In addition, NPY might influence consumption behaviors through reward effects [Ehlers et al., 1998a; Tecott and Heberlein, 1998]. NPY is expressed in the amygdala and nucleus accumbens, structures of the mesolimbic dopamine system that are thought to mediate the rewarding aspects of food, alcohol and certain drugs [Tecott and Heberlein, 1998; Ault et al., 1993; Jewett et al., 1992]. Despite the circumstantial evidence from animal models, no studies on the role of NPY in the regulation of alcohol consumption in humans have yet been published.

A novel finding of a common polymorphism in the signal peptide of NPY was recently reported [Karvonen et al., 1998]. After screening the entire coding region of the NPY gene for sequence variants, a thymidine(1128) to cytosine(1128) polymorphism(T1128C) was identified, resulting in a substitution of Leu(7) to Pro(7) in the signal peptide part of preproNPY. The Pro (7) in NPY showed a strong association with elevated serum cholesterol levels [Karvonen et al., 1998].

In the present study we found that the Leu (7) to Pro (7) polymorphism in NPY is related to the level of alcohol consumption in an unselected male population sample from the Kuopio Ischemic Heart Disease Risk Factor Study (KIHD) [Salonen, 1988; Lakka et al., 1994].

SUMMARY OF THE INVENTION

According to one aspect, this invention concerns a method for diagnosing a person's susceptibility for having a risk for the development of alcoholism, said method comprising determining whether said subject has a polymorphism in the signal peptide part of the human preproNPY, said polymorphism comprising the subsitution of the position 7 leucine for proline in the signal peptide part of said preproNPY, said polymorphism being indicative of a risk for the development of alcoholism.

According to a second aspect, the invention concerns a method for treating a person, diagnosed for having a risk for the development of alcoholism, for the prevention of developing alcoholism or for alleviating or curing of alcoholism, comprising administering to said person an effective amount of an agent counteracting the influence of the mutated NPY gene.

According to a third aspect, the invention concerns a method for treating a person, diagnosed for having a risk for the development of alcoholism, for the prevention of developing alcoholism or for alleviating or curing of alcoholism, comprising subjecting the person to specific gene therapy aimed to repair the mutated NPY signal peptide sequence.

According to a fourth aspect, the invention concerns a method to investigate or screen pharmaceuticals or genetic aims useful in the prevention or treatment of alcoholism, by using an animal model including a transgenic animal which carries a human DNA sequence comprising a nucleotide sequence encoding a prepro-neuropeptide Y (preproNPY) or part thereof encoding mature human NPY peptide, where the leucine amino acid in position 7 of the signal peptide part of said preproNPY i) is unchanged or ii) has been replaced by proline.

According to a fifth aspect, the invention concerns a method to investigate or screen pharmaceuticals or genetic aims useful in the prevention or treatment of alcoholism, by using an animal model including a transgenic animal, which carries a DNA sequence comprising a nucleotide sequence encoding otherwise normal mouse NPY sequence or part thereof encoding mature mouse NPY peptide, but in which the nucleotide sequence encoding the mouse signal peptide is replaced by human signal peptide sequence encoding either normal or mutated human signal peptide.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1a illustrates schematically the molecular structure of the human NPY gene, the preproNPY peptide and the mature NPY peptide,

Figure 1b shows the nucleotide sequence of the human NPY gene. Upper case indicates exonic sequences and lower case intronic sequences. Genbank accession numbers are given in parenthesis. The arrow shows the position in which thymidine (T) of the normal gene is replaced by cytosine (C) to give the mutant gene. The underlined sequence in Exon 2 is the sequence encoding the signal peptide of 28 amino acids (Exon 1 is SEQ ID NO:1, exon 2 is SEQ ID NO:2, exon 3 is SEQ ID NO:3 and exon 4 is SEQ ID NO:4), and

Figure 1c shows the nucleotide sequence of the human preproNPY mRNA (SEQ ID NO:5, with the protein sequence set forth in SEQ ID NO:6). The arrow shows the position in which thymidine (t) of the normal mRNA is replaced by cytosine (c) to give the mutant mRNA.

DETAILED DESCRIPTION OF THE INVENTION

Neuropeptide Y (NPY) is a 36-amino-acid neurotransmitter widely present in the central and peripheral nervous systems. NPY has multiple actions, which control body energy balance and cardiovascular function. We have recently demonstrated that the subjects having Pro7 in the signal peptide of NPY have higher serum cholesterol and apolipoprotein B levels when compared to individuals having wildtype (Leu7/Leu7) signal peptide sequence. Neuropeptide Y (NPY) plays an important role in the hypothalamic regulation of food intake and energy balance. According to recent findings in animals, NPY also appears to be a potent regulator of alcohol consumption. We used recently identified Leu (7) to Pro (7) polymorphism in the signal peptide part of NPY to investigate wheter the NPY system is associated with alcohol consumption in humans. The subjects (N=889) were an ethnically homogenous, unselected population sample of middle-aged men from Eastern Finland. The gene variant producing Pro (7) substitution was associated with a 34 % higher average alcohol consumption, even following adjustment for a number of covariates (p=0.03). The proportion of heavy drinkers (over 230 grams of ethanol/week) was also somewhat higher in this group (13.1 % vs. 8.2 %, p=0.10). Our study provides the first evidence that alcohol preference in humans is likely to be regulated by the NPY system.

The DNA sequence or the mutant signal peptide or said peptide associated with any other cleavage product of preproNPY can be used for screening a subject to determine if said subject is a carrier of a mutant NPY gene.

The determination can be carried out either as a DNA analyse according to well known methods, which include direct DNA sequencing of the normal and mutated NPY gene, allele specific amplification using the polymerase chain reaction (PCR) enabling detection of either normal or mutated NPY

sequence, or by indirect detection of the normal or mutated NPY gene by various molecular biology methods including e.g. PCR- single stranded conformation polymorphism (SSCP)-method or denaturing gradient gel electrophoresis (DGGE). Determination of the normal or mutated NPY gene can also be done by using restriction fragment length polymorphism (RFLP)-method, which is particularly suitable for genotyping large number of samples.

The determination can also be carried out at the level of RNA by analysing RNA expressed at tissue level using various methods. Allele spesific probes can be designed for hybridization. Hybridization can be done e.g. using Northern blot, RNase protection assay or in situ hybridization methods. RNA derived from the normal or mutated NPY gene can also be analysed by converting tissue RNA first to cDNA and thereafter amplifying cDNA by an allele spefic PCR-method and carrying out the analysis as for genomic DNA as mentioned above.

Alternatively, the determination can be carried out as an immunoassay where a sample is contacted with an antibody capable of binding the signal peptide or said peptide associated with any other cleavage product of preproNPY.

Antibodies can be raised against normal or mutated preproNPY or more specifically against normal or mutated signal peptide part of the NPY. The production of antibodies can be done in experimental animals in vivo to obtain polyclonal antibodies or in vitro using cell lines to obtain monoclonal antibodies.

A person diagnosed for having a risk for the development of alcoholism can be treated for the prevention of developing said condition by administering to said person an effective amount of an agent counteracting the influence of the

mutated NPY gene. This can be done by specific gene therapy aimed to repair the mutated NPY sequence, or by administering pharmacotherapies, which are aimed to modulate synthesis, release or metabolism of the endogenous NPY, or to interact in a specific manner at NPY target sites by modulating effects of NPY with specific NPY receptor proteins. Currently, five different subtypes of NPY receptors have been cloned and characterized (Y1-Y5 receptors) and drug molecules specifically interacting with these NPY receptors have been synthesized. The pharmacotherapy described is not limited to only these named receptors or mechanisms, but also covers other NPY receptors and related mechanisms to be discovered including the secretion of NPY.

The influence of the mutated NPY gene in a patient can be counteracted by using an antisense therapy or gene switching or replacement, which includes targeted correction of disease-related mutation or site-directed inactivation of the mutant allele by homologous recombination.

The antisense therapy refers to methods designed to impair translation through direct interactions with target messenger RNA (mRNA). This can be accomplished by applying a targeted oligonucleotide, which forms Watson-Crick base pairs with the messenger RNA whose function is to be disrupted. The inhibition of gene expression by antisense oligonucleotide depends on the ability of an antisense oligonucleotide to bind a complementary mRNA sequence and prevent the translation of the mRNA. It is possible to correct a single mutant base in a gene by using an oligonucleotide based strategy (Giles et al., 1995; Schwab et al., 1994; Yoon et al., 1996). A short, 7 or 8 bases, oligonucleotide is enough to posses an antisense activity and specificity, which depends greatly on the flanking sequences of the target RNA. Binding should be enough to promote stable binding and RNase H – mediated cleavage.

The influence of the mutated NPY gene is preferably counteracted by using a short, allele specific oligonucleotide, which includes the sequence of mutated part: ...cga ct/cg ggg.... This can be accomplished by using oligonucleotides of various lengths, but all recognizing the mutated base sequence. According to the predicted secondary structure of the preproNPY mRNAs (Schemes 1 and 2), the best target sequence is between –9 and +2 bases around the mutation i.e. sequence targeting to 3'-ac aag cga ctg g-5'. This sequence contains 'bulbs' which are known to enhance the binding of oligonucleotide to the target mRNA.

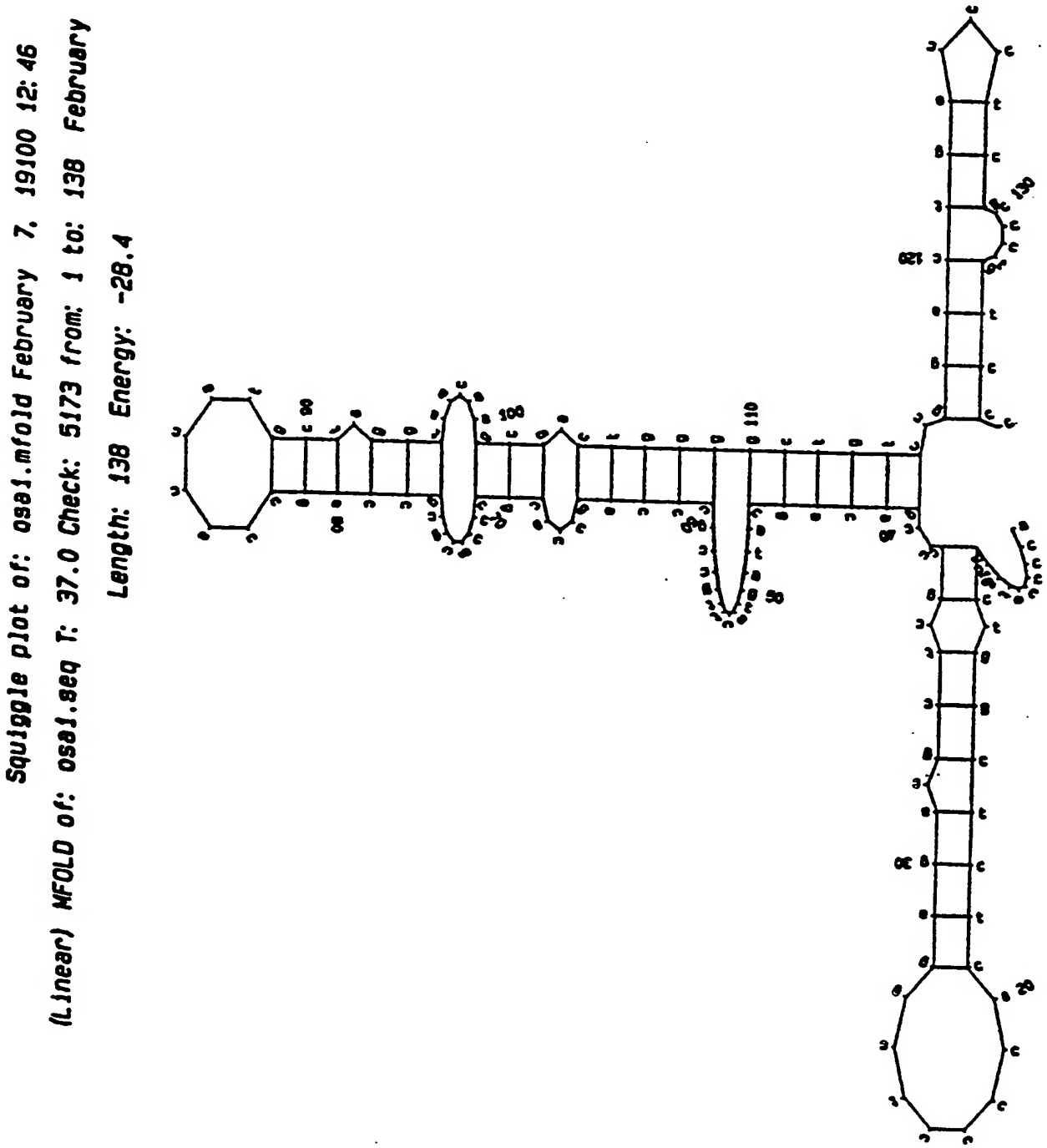
It is possible to use unmodified oligonucleotides, but to increase their stability, nuclease resistance, and penetration to the nucleus, several modifications of oligonucleotide can be used. A relatively large number of modified pyrimidines have been synthesized, mainly C-2, C-4, C-5, and C-6 sites, and incorporated into nucleotides. Also purine analogs can be synthesized and incorporated into oligonucleotides. The 2' position of the sugar moiety, pentofuranose ring, is substituted with methoxy, propoxy, O-alkoxy or methoxyethoxy groups. A new backbone for oligonucleotides that replace the phosphate or the sugar-phosphate unit has been made, like C-5 propynylpyrimidine-modified phosphothioate oligonucleotides. Also chimeric oligonucleotides with 5'- and 3'-ends are modified with internucleotide linkages, like methylphosphorothioate, phosphodiester, or methylphosphonate can be used. A relatively new technique is conformationally restricted LNA (locked nucleic acid) oligonucleotides and peptide nucleic acids. Bioengineered ribozymes are structurally different, but their specificity also relay on the recognition of the targeted mRNA sequence.

Gene replacement or gene switching techniques inactivate the mutated gene sequence and introduce a corrected one. This can be accomplished by

transfecting exogenous gene with normal coding sequence and blocking mutant coding sequence with antisense oligonucleotide. Also a technique with only introducing a corrected normal sequence without disrupting the mutated sequence could be use. This could be used in heterozygous cells i.e. cell carrying one normal allele and one mutated allele resulting in an overexpression of normal alleles. Also homozygous mutant cells could be treated resulting in a dominant positive –effect i.e. the normal allele is expressed in higher degree than the mutant allele.

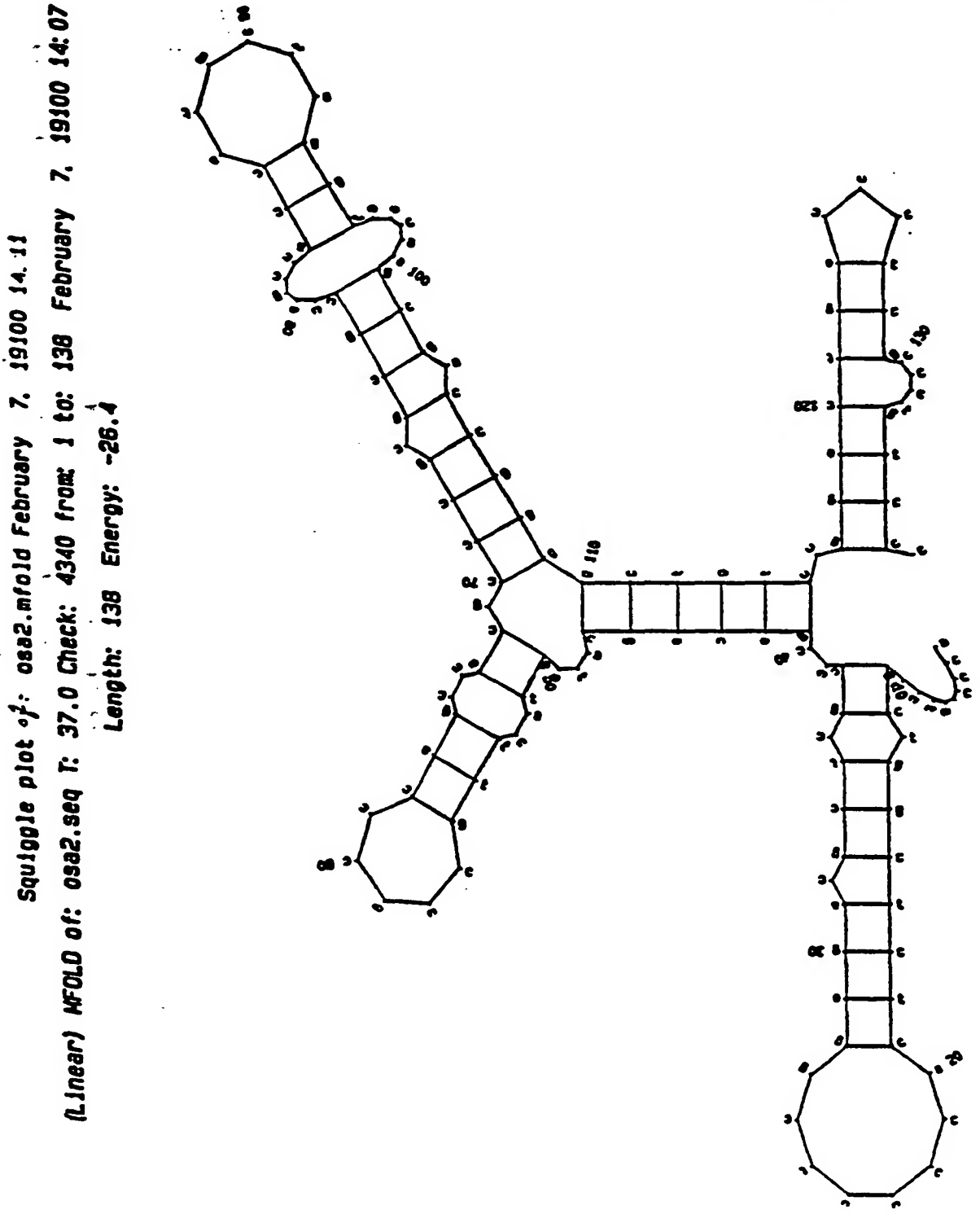
Scheme 1

Predicted secondary structure of preproNPY mRNA. The Scheme shows the predicted structure of the 5' end (1 to 138 bases) of the full preproNPY mRNA sequence published in GenBank Accession No. K01911. The secondary structure was predicted by using the MFOLD program of the Genetics Computer Group of the University of Wisconsin.



Scheme 2

Predicted secondary structure of preproNPY mRNA. The Scheme shows the predicted structure of the 5' end (1 to 138 bases) of the full preproNPY mRNA sequence published in GenBank Accession No. K01911. The secondary structure was predicted by using the MFOLD program of the Genetics Computer Group of the University of Wisconsin. The mutated base T to C is base number 106.



Influence of the mutated NPY sequence on the funtion of NPY gene can be investigated in transgenic animals. A transgenic animal can be generated using targeted homologous recombination methodology. Both normal and mutated sequence of human NPY signal peptide (or any DNA sequence comprising a nucleotide sequence encoding a prepro-neuropeptide Y (preproNPY) or part thereof encoding the amino acid sequence of the mature mouse or human mature NPY peptide, where either i) the leucine amino acid in position 7 of the signal peptide part of said preproNPY has been replaced by proline or ii) the leucine amino acid in position 7 of the signal peptide part of said preproNPY is unchanged) will be introduced into the sequence of NPY gene to replace the endogenous signal peptide sequence. Under these conditions, the endogenous NPY gene functions otherwise normally, but the synthesis of the preproNPY is regulated by either normal or mutated human NPY signal peptide sequence. This transgenic model can be used to investigate in a very specific manner the physiological importance of the mutated NPY gene. It also will provide an ideal preclinical model to investigate and screen new drug molecules, which are designed to modify the influence of the mutated NPY gene.

The invention is described more in detail in the following experiments.

EXPERIMENTAL

Materials and methods

Study subjects

The study population consisted of the participants of the Kuopio Ischemic Heart Disease Risk Factor Study (KIHD), a population-based epidemiologic study that was launched in the 1980's to investigate previously unestablished

risk factors for myocardial infarction, progression of atherosclerosis, and other major health outcomes in middle-aged men [Salonen, 1988; Lakka et al., 1994]. The study protocol has been approved by the Research Ethics Committee of the University of Kuopio, and all participants gave a written informed consent to participate in KIHD.

The total sample of the KIHD study consists of 2,682 men who were recruited in two cohorts. The present study is based on the second cohort, which is an age-stratified sample of 42-, 48, 54-, and 60 year-old men (N=1,516, participation rate 82.6 %) enrolled in the study between 1986 and 1989. A DNA sample was obtained for 1,137 men who were free from coronary heart disease at baseline.

Assessment of alcohol consumption

A self-report quantity-frequency questionnaire [Kauhanen et al., 1997a; Kauhanen et al., 1997b] was used to record the level of alcohol use. The average weekly consumption of alcohol in pure ethanol (grams/week) was calculated based on the known alcoholic content of each beverage type and the reported doses and frequencies of drinking sessions. We further calculated the proportion of heavy users consisting of those whose average daily consumption exceeded 3 standard doses (>230 grams of ethanol/week). One dose is a 12 fl ounze bottle of beer, 12 cl of wine, or a 4 cl shot of hard liquor. Serum gamma-glutamyltranspeptidase (GGT) and mean corpuscular volume (MCV) were determined from baseline blood samples as biomarkers of excessive alcohol use. These biochemical measures were checked to see if any of the genotype groups showed biochemical signs of actual alcohol abuse.

Men who told they had not been drinking at all for at least 12 months were determined as abstainers (N=123, a total 12.1 %). Since abstainers are a heterogenous group consisting of those who have quit because of health problems, they were excluded from final analyses.

Covariates

A number of sociodemographic, behavioral and medical characteristics were assessed according the KIHD protocol as described earlier [Salonen, 1988; Lakka et al., 1994; Kauhanen et al., 1997a]. Age, place of living (urban/rural), marital status, educational level, current income, history of smoking in cigarette-years, and history of diagnosed chronic diseases and conditions (ischemic heart disease, diabetes, stroke, cancer, liver disease, mental disorder) and history of trauma were recorded by a questionnaire and double-checked in the clinical interview. The data were used to examine the possible effect of confounding in the observed relationship.

Genotype analysis

PreproNPY genotype was determined by restriction fragment length polymorphism (RFLP) analysis from DNA extracted from the subjects' peripheral blood by an investigator unaware of phenotype. Briefly, the polymorphism appears as a thymidine(1128) to cytosine(1128) substitution generating a Bsi EI restriction site, which was used to genotype the subjects for the Leu7Pro polymorphism, as described previously [Karvonen et al., 1998]. The PCR products were digested by Bsi EI [New England Biolabs, Inc. Beverly, MA, USA] and digestions were analyzed by electrophoresis on 2% agarose gel.

Statistical analyses

The allelic frequency distribution was tested for Hardy-Weinberg equilibrium by the X^2-test. Statistical differences in the mean weekly alcohol consumption between the genotype groups were examined in the analysis of variance. Age and other covariates were adjusted for in analysis of covariance. The proportion of heavy drinkers in the genotype groups was compared using a chi-square test. P-values less than 0.05 obtained from the statistical tests were interpreted as statistically significant. Statistical computations were performed using the SPSS software for IBP RS/6000 [SPSS for Unix, SPSS Inc., Chicago, USA].

Results

The analysis of the Leu(7)-to Pro(7) polymorphism in the signal peptide part of the pre-pro-NPY and complete information on alcohol use was available for 889 alcohol using men. Of these, 790 (88.9 %) were genotyped as Leu(7)/Leu(7) homozygous, a total of 95 (10.7 %) were Leu(7)/Pro(7) heterozygous, and 4 (0.4 %) were Pro(7)/Pro(7) homozygous. The allele frequencies were 94.2 % (Leu) and 5.8 % (Pro). All men carrying either one or two Pro(7) alleles were pooled for further analyses. The study population was in Hardy-Weinberg equilibrium (chi^2=0.585, 1 d.f., p=0.44).

Table I shows sociodemographic and behavioral background characteristics, and the proportion of men with diagnosed diseases in the two NPY genotype groups. There were no differences in the serum level of gamma glutamyl transpeptidase (GGT) or mean corpuscular volume (MCV) between genotypes. The means and standard deviations of GGT were 29.0 U/l (SD 29.4) among Leu(7)/Leu(7) homozygotes and 29.7 U/l (26.0) among those with Pro(7) (p= 0.83). For MCV the means and standard deviations were 92.0 fl (SD 4.52) and 92.0 fl (SD 4.0), respectively (p= 0.93).

The alcohol consumption in grams of pure ethanol per week is presented in Table II. Both the unadjusted mean consumption and the covariate-adjusted consumption were significantly (33 percent) higher among men who were carriers of Pro(7). The proportion of heavy drinkers (men who reported drinking on average over 230 grams of ethanol/week or over 3 standard doses/day) was also higher among men with a Pro(7) substitution (13.1 % vs. 8.2 %) (p=0.10).

Table I. Means (standard deviations) and proportions of background variables by the NPY genotype.

	Leu(7) homozygotes (N=790)	Pro(7) carriers (N=99)
Age (years)	56.1 (SD 6.7)	56.1 (SD 6.9)
Living in rural area	21.8 %	27.0 %
Annual income (US $)	24,130 (SD 15,918)	26,862 (SD 14,771)
Educational level (1= low, 7= high)	2.05 (SD 1.75)	2.13 (SD 1.92)
Married	87.1 %	86.9 %
Cigarette smoking (pack-years)	141.3 (SD 292.1)	147.4 (SD 311.7)
Ischemic heart disease	21.1 %	13.1 %
Diabetes	5.6 %	5.1 %
History of cancer	2.4 %	5.1 %
History of stroke	2.6 %	1.0 %
Liver disease	0.4 %	1.0 %
History of mental disorder	4.6 %	6.1 %
History of trauma	10.4 %	10.2 %

Table II. Mean weekly alcohol consumption in pure ethanol according to the NPY genotype.

	Leu (7) homozygotes (N=790)	Pro(7) carriers (N=99)	P-value
Unadjusted mean alcohol consumption (g/wk)	86.3 (SD 127.6)	115.0 (SD 173.9)	0.030
Mean alcohol consumption (g/wk) adjusted for all covariates*	86.4	114.7	0.035

*Adjusted for age, place of living, education, income, marital status, smoking history in cigarette-years, history of ischemic heart disease, diabetes, cancer, stroke, liver disease, mental disorder and trauma.

Discussion

We observed an increased alcohol consumption in a population sample of middle-aged men who were homozygous or heterozygous for the variant allele in a common polymorphism substituting Leu(7) by Pro(7) in the signal peptide part of neuropeptide Y (NPY). Presence of Pro(7) was associated with approximately one-third (33 %) higher average consumption of ethanol as compared to homozygous subjects with the Leu(7)/Leu(7) genotype. The proportion of heavy consumers who report using over 230 grams of ethanol/week was also higher among men with Pro(7) mutation, although this difference did not reach statistical significance due to smaller numbers of subjects.

Our study is the first one to show a relationship between a common NPY polymorphism and alcohol use in humans. The results are in line with the findings from a number of recent animal studies [Ehlers et al., 1998a; Ehlers et al., 1998b; Thiele et al., 1998; Cockerill, 1998; Tecott and Heberlien, 1998] that have shown an inverse relationship between levels of NPY in central nervous system and preference for alcohol. Mice with no neuropeptide Y are especially fond of alcohol and less sensitive to the effects of ethanol as compared to mice that have normal or extra neuropeptide Y levels [Thiele et al., 1998], and alcohol-preferring rats have lower levels of NPY in amygdala, hippocampus, and frontal cortex [Ehlers et al., 1998a].

The allele frequencies in our study were close to those seen earlier in two Finnish populations [Karvonen et al., 1998]. It is highly unlikely that the observed association could be due to a stratification error in sampling, or population admixture, since Finns are known to be genetically a rather homogenous population.

Many sociodemographic factors are known determinants of alcohol use. In our study the social background among men with and without Pro(7) was similar. The two groups were of the same age and had similar educational background. Slightly more men with Pro(7) were living in rural communities, and this group also had a little higher average income. Smoking history was similar in both groups. It was somewhat unexpected to observe a higher prevalence of ischemic heart disease history among the Leu (7) homozygotes, since earlier findings have shown this genotype to associate with lower serum levels of total and LDL choclesterol [Karvonen et al., 1998]. Adjustment for all these variables in the multivariate model did not affect the observed association between the NPY polymorphism and alcohol consumption, indicating that these variables did not confound the findings.

There are several physiologically plausible mechanisms that can explain the effect of NPY on alcohol use. NPY is an inhibitory neuromodulator that acts widely in the brain. The NPY receptors couple to heterotrimeric G proteins that inhibit production of cyclicAMP [Thiele et al., 1998; Lamme, 1995], so it is possible that NPY inhibits cAMP production in response to alcohol, thus limiting alcohol intake. Central administration of NPY reduces anxiety, and NPY-deficient mice score high on measures of anxiety [Heilig et al., 1992; Palmiter et al., 1998]. The development of alcohol preference may in part depend on the relative lack of tension-reducing NPY.

Chronic exposure to ethanol in rats affects NPY levels in hypothalamus in a fashion similar to food restriction [Ehlers et al., 1998a]. NPY has an important role in the hypothalamic regulation of energy balance by potently stimulating short-term food intake [Clark et al., 1985; Levine and Morley, 1985; Stanley and Leibowitz, 1985]. Centrally administered NPY also increases the expression of lipoprotein lipase mRNA and enhances the enzyme activity in white fat favoring lipid storage [Billington et al., 1991; Billington et al., 1994]. Thus, NPY might unspecifically affect the consummatory behaviors regarding both food intake and alcohol drinking. However, there is a lack of NPY transgene expression in the arcuate nucleus of the hypothalamus, a region thought to regulate food intake [Thiele et al., 1998; Palmiter et al., 1998]. This indicates that the effects of NPY on alcohol use are probably not mediated through similar mechanisms as with food and calorie intake.

To our knowledge, there is only one earlier human study examining the possible relationship between neuropeptide Y and addictions. Roy and coworkers [1990] did not observe significant differences of cerebrospinal fluid (CFS) levels of NPY between male alcoholics and normal controls. Alcoholics, however, do not represent the population at large. It is also

unclear, whether the CFS levels of NPY reflect the activity of this peptide in the physiologically important locations of the central nervous system.

Plasma NPY is derived from sympathetic nerve terminals and thus levels of NPY in plasma reflect the level of sympathetic activity [Lundberg et al., 1990]. Significant positive correlations have been observed between levels of NPY and corticotropin-releasing hormone, somatostatin, and growth hormone in cerebrospinal fluid [Roy et al., 1990]. Based on these studies and on our findings, further research on the possible sympathetic nervous system mechanisms in drinking behavior is warranted.

It will be appreciated that the methods of the present invention can be incorporated in the form of a variety of embodiments, only a few of which are disclosed herein. It will be apparent for the specialist in the field that other embodiments exist and do not depart from the spirit of the invention. Thus, the described embodiments are illustrative and should not be construed as restrictive.

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CLAIMS

1. A method for diagnosing a person's susceptibility for having a risk for the development of alcoholism, said method comprising determining whether said subject has a polymorphism in the signal peptide part of the human preproNPY, said polymorphism comprising the subsitution of the position 7 leucine for proline in the signal peptide part of said preproNPY, said polymorphism being indicative of a risk for the development of alcoholism.

2. The method according to claim 1 wherein said polymorphism in the signal peptide part of the human preproNPY at said subject is determined by subjecting a position 7 allele specific oligonucleotide probe to a sample from said subject, said sample comprising a target polynucleotide of said preproNPY.

3. The method according to claim 1 wherein said polymorphism in the signal peptide part of the human preproNPY at said subject is determined by immunoassay where a sample from said subject is contacted with an antibody capable of binding the signal peptide or said NPY peptide associated with any other cleavage product of preproNPY.

4. A method for treating a person, diagnosed for having a risk for the development of alcoholism according to claim 1, 2 or 3, for the prevention of developing alcoholismor for alleviating or curing alcoholism , comprising administering to said person an effective amount of an agent counteracting the influence of the mutated NPY gene.

5. The method according to claim 4 wherein said agent is a pharmaceutical aimed to modulate synthesis, secretion or metabolism of the endogenous

NPY, or to interact in a specific manner at NPY target sites by modulating effects of NPY with specific NPY receptor proteins.

6. The method according to claim 4 wherein said agent is a pharmaceutical aimed to modulate gene expression of normal or mutated NPY gene.

7. A method for treating a person, diagnosed for having a risk for the development of alcoholism according to claim 1, 2 or 3, for the prevention of developing alcoholism or for alleviating or curing alcoholism, comprising subjecting the person to specific gene therapy aimed to repair the mutated NPY sequence.

8. A method to investigate or screen pharmaceuticals or genetic aims useful in the prevention or treatment of alcoholism, by using an animal model including a transgenic animal which carries a human DNA sequence comprising a nucleotide sequence encoding a prepro-neuropeptide Y (preproNPY) or part thereof encoding mature human NPY peptide, where the leucine amino acid in position 7 of the signal peptide part of said preproNPY i) is unchanged or ii) has been replaced by proline.

9. A method to investigate or screen pharmaceuticals or genetic aims useful in the prevention or treatment of alcoholism, by using an animal model including a transgenic animal, which carries a DNA sequence comprising a nucleotide sequence encoding otherwise normal mouse NPY sequence or part thereof encoding mature mouse NPY peptide, but in which the nucleotide sequence encoding the mouse signal peptide is replaced by human signal peptide sequence encoding either normal or mutated human signal peptide.

SEQUENCE LISTING

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